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ORIGINAL ARTICLE

Reversible and regionally selective downregulation of brain cannabinoid CB_1 receptors in chronic daily cannabis smokers

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Chronic cannabis (marijuana, hashish) smoking can result in dependence. Rodent studies show reversible downregulation of brain cannabinoid CB_1 (cannabinoid receptor type 1) receptors after chronic exposure to cannabis. However, whether downregulation occurs in humans who chronically smoke cannabis is unknown. Here we show, using positron emission tomography imaging, reversible and regionally selective downregulation of brain cannabinoid CB_1 receptors in human subjects who chronically smoke cannabis. Downregulation correlated with years of cannabis smoking and was selective to cortical brain regions. After ~4 weeks of continuously monitored abstinence from cannabis on a secure research unit, CB_1 receptor density returned to normal levels. This is the first direct demonstration of cortical cannabinoid CB_1 receptor downregulation as a neuroadaptation that may promote cannabis dependence in human brain.
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Introduction

Cannabis is the most widely used illicit drug in the world[1] and causes multiple health problems.[2] Chronic cannabis smoking can lead to tolerance[3–5] and withdrawal symptoms,[6,7] the two hallmarks of dependence. In human brain, the actions of the main psychoactive component of cannabis, Δ^9-tetrahydrocannabinol (Δ^9-THC), are mediated via cannabinoid CB_1 (cannabinoid receptor type 1) receptors.[8,9] CB_1 receptors are widely distributed in human brain, and highest receptor densities are found in basal ganglia, hippocampus, cingulate cortex and the molecular layer of cerebellum.[10,11] CB_1 receptors are primarily located presynaptically, where they inhibit release of other neurotransmitters, such as glutamate and γ-amino butyric acid.[12,13] However, the exact role of CB_1 receptors in the development of tolerance and withdrawal in humans is unclear.

In rodent brain, downregulation of CB_1 receptor signaling is thought to underlie tolerance.[14] That is, chronic exposure to Δ^9-THC and other cannabinoid agonists causes a reduction in the number and signaling efficiency of CB_1 receptors as a homeostatic response.[15–21] These changes closely parallel development of tolerance to typical cannabinoid effects, such as decreased motility and impaired memory.[15] The magnitude and rate of downregulation are region dependent. The downregulation is larger and occurs more rapidly in cortical regions, such as hippocampus and cerebellum, than in subcortical regions, such as basal ganglia and midbrain.[15] In addition, downregulation is reversible upon abstinence: this reversal is more rapid in striatum and midbrain than in cortical regions.[20]

Whether chronic cannabinoid exposure downregulates CB_1 receptor signaling in human brain is unknown, although such downregulation has been hypothesized to underlie the development of tolerance to some effects of cannabis in heavy smokers.[4] Lack of methods to accurately quantify CB_1 receptors in human brain *in vivo* has hindered progress in answering this question. We recently developed a method to quantify cannabinoid CB_1 receptors in human brain with positron emission tomography (PET) using a novel inverse agonist radioligand, [^{18}F]FMPEP-d_2.[22,23] This radioligand has high affinity and selectivity for the CB_1 receptor and, in monkey brain, the vast majority of the signal represents specific binding to CB_1 receptors.[22] In human brain,

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[^{18}F]FMPEP-d_2 has high brain uptake, and binding can be reliably measured as the distribution volume (V_T), which is proportional to receptor density.[22]

To determine whether heavy cannabis smoking downregulates cannabinoid CB_1 receptors in human brain, we measured CB_1 receptors using PET and [^{18}F]FMPEP-d_2 in chronic daily cannabis smokers ($N=30$ subjects) and in control subjects with minimal lifetime exposure to cannabis ($N=28$ subjects). Cannabis smokers were scanned twice: immediately after chronic daily cannabis smoking, and after $\sim$4 weeks of abstinence on a monitored unit. Based on animal experiments, we hypothesized that CB_1 receptor binding is decreased in chronic daily cannabis smokers at baseline but recovers to normal levels after abstinence.

Subjects and methods

The National Institutes of Health (NIH) Central Nervous System Institutional Review Board approved the protocol and the consent forms. Written informed consent was obtained from all subjects.

Study design
We admitted male chronic daily cannabis smokers to a closed and monitored in-patient research unit for $\sim$4 weeks. We imaged cannabis smokers with PET and [^{18}F]FMPEP-d_2 at two time points—namely, on the day following an evening admission and after $\sim$4 weeks of abstinence. The time of first PET scan was chosen to maximize the effect size of the hypothesized downregulation while avoiding both acute intoxication and withdrawal symptoms. Healthy control male subjects underwent a single PET scan. To examine downregulation in cannabis smokers at baseline, we compared V_T of [^{18}F]FMPEP-d_2 at baseline between cannabis smokers and control subjects (between-subject comparison). To examine whether downregulation is reversible upon abstinence, we compared V_T before and after abstinence in cannabis smokers (within-subject comparison).

Subjects
A total of 30 male subjects who smoked cannabis daily but who were not seeking treatment were recruited by the National Institute on Drug Abuse (Table 1). Participants smoked an average of 10 ± 6 joints or blunts per day (range 1–30) for 12 ± 7 years (range 4–37). Age of first cannabis smoking was 15 ± 3 years (range 6–22). The participants underwent history and physical examination as well as laboratory tests of blood and urine to ensure that they were free of current somatic and psychiatric illness and did not currently abuse drugs other than cannabis. Urine drug tests were positive for cannabinoids in these subjects at admission. In addition, 24 cannabis smokers (80%) also smoked tobacco.

Control male subjects ($N=28$) were recruited by the National Institute of Mental Health in Bethesda, MD. All subjects were free of somatic and psychiatric illness as confirmed by history, physical examination, electrocardiogram and blood and urine tests. Urine samples were negative for cannabinoids, opiates, amphetamines, cocaine metabolites and benzodiazepines. Control subjects had <10 lifetime exposures to cannabis and no use in the preceding 3 months. One control subject (4%) smoked tobacco. Control subjects used slightly less alcohol than cannabis smokers (Table 1).

Residential stay and assessments in cannabis smokers
Cannabis smokers were admitted to the Johns Hopkins Behavioral Pharmacology Research Unit (BPRU) in Baltimore, MD. BPRU has a secure, continuously monitored research unit with nursing personnel present 24 h a day. Subjects were not allowed to leave the unit or receive visitors during their stay to exclude illicit drug use. Subjects were transported to the NIH Clinical Center for PET imaging on the day following an evening admission and again after $\sim$4 weeks, as well as once for magnetic resonance imaging of the brain. Urine samples were tested before leaving the unit, at arrival to NIH and after returning to the unit to ensure abstinence from drugs. Psychological and

Table 1 Demographic, clinical and radiochemical information of the study participants

	Cannabis smokers	*Control subjects*	P-*value*
Number of subjects	30	28	
Age (years)	28 ± 8	32 ± 10	0.096
BMI (kg m^{-2})	24 ± 4	27 ± 5	0.022
Tobacco smokers/nonsmokers (*N*)	24/6	1/27	0.001
Amount of alcohol use (drinks per week)	5 ± 7	2 ± 2	0.041
Amount of cannabis smoking (joints per day)	10 ± 6	NA	NA
Duration of cannabis smoking (years)	12 ± 7	NA	NA
Age at first cannabis smoking (years)	15 ± 3	NA	NA
Injected activity of [^{18}F]FMPEP-d_2 (MBq)	175 ± 17	181 ± 4	0.051
Injected mass of [^{18}F]FMPEP-d_2 (μg)	0.84 ± 0.45	0.74 ± 0.24	0.273
Fraction of free [^{18}F]FMPEP-d_2 in plasma (%)	0.42 ± 0.2	0.40 ± 0.2	0.733

Abbreviations: BMI, body mass index; NA, not applicable.
Values are number, mean ± s.d. or range.

physical symptoms of cannabis withdrawal were measured daily with 24 five-point Likert scale items.[6] Cannabis craving was measured daily with the 12-point Marijuana Craving Questionnaire.[24]

PET and measurement of parent radioligand in arterial plasma

[^{18}F]FMPEP-d_2 was prepared as described previously[23] and in detail in our Investigational New Drug Application 105 198, submitted to the US Food and Drug Administration (available at http://pdsp.med.unc.edu/snidd/). The radioligand was obtained in high radiochemical purity (>99%) and had a specific radioactivity of 110 ± 43 MBq nmol^{-1} at the time of injection.

After intravenous injection of [^{18}F]FMPEP-d_2 (Table 1), images were acquired for 120 min using an Advance camera (GE Healthcare, Milwaukee, WI, USA) as previously described.[22] Blood samples were drawn from the radial artery at 15 s intervals until 2 min, then at 3 and 5 min, followed by 3 to 6 ml samples at 10, 15, 20, 30, 45, 60, 75, 90, 105 and 120 min. Plasma time-activity curve was corrected for the fraction of unchanged radioligand by radio-high-performance liquid chromatography separation,[25] and the plasma free fraction was measured using ultrafiltration.[26]

PET images were analyzed by applying a template of volumes of interest[27] as implemented in PMOD, version 3.0 (PMOD Technologies, Zurich, Switzerland),[28] in the standard stereotactic space[29] (see Supplementary Material for details). Distribution volume (V_T) was estimated according to the two-tissue compartmental model[30] with concentration of parent radioligand in plasma as input function, using PMOD, as previously described.[22]

Statistical analysis of V_T *data*

Data were analyzed using SPSS Statistics 17.0 for Windows (Release 17.0.0, copyright SPSS, 1993–2007, Chicago, IL, USA). V_T had normally distribution (Shapiro–Wilk test) and homogeneous variance across groups (Levene's test). Behavioral data were non-normally distributed. To test whether CB_1 receptors are decreased in cannabis smokers at baseline, we applied a repeated measures analysis of variance with group status as a between-subject factor and brain region as a within-subject factor. Body mass index (BMI) entered the model as a covariate as it was different between the groups (Table 1). Correlations with clinical variables were assessed with the non-parametric Spearman's correlation coefficients (ρ) and BMI-adjusted V_T. For correlations and visualizations, V_T was adjusted for each subject to the average BMI in the whole sample as follows: $V_{T\ adjusted} = V_{T\ observed} + b \times (BMI_{average} - BMI_{observed})$, where b is the slope of the regression line between V_T and BMI in control subjects. To test whether CB_1 receptors increased after abstinence from cannabis, we applied a repeated measures analysis of variance with repetition (before and after abstinence) and brain region as within-subject factors. The P-values <0.05 were considered statistically significant.

Voxel-wise analysis of V_T

To confirm results from volume-of-interest analysis and to explore regional specificity of findings, we compared baseline parametric V_T maps between groups at voxel level using SPM5 (Wellcome Trust Centre for Neuroimaging, University College London, London, UK) (see Supplementary Material for details).

Results

At baseline, V_T of [^{18}F]FMPEP-d_2 was lower in cannabis smokers than in control subjects in a region-specific manner (group × region interaction: $F = 6.5$, $P = 0.0001$). BMI-adjusted V_T was ~20% lower in neocortex and limbic cortex, but not in other brain regions (basal ganglia, midbrain, thalamus, pons, or cerebellum; Figures 1 and 2). This finding was confirmed by an independent statistical parametric

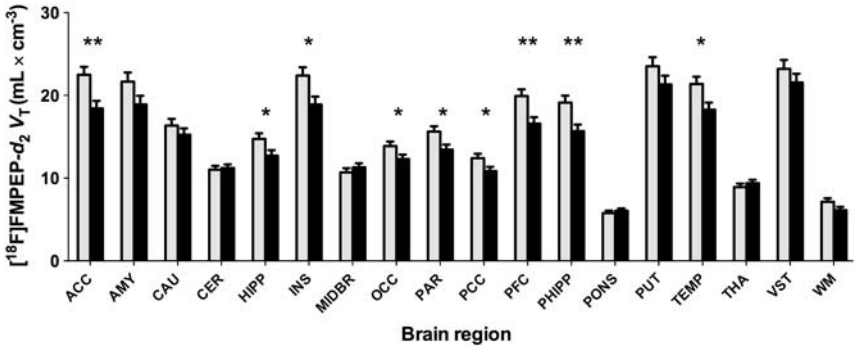

Figure 1 V_T of [^{18}F]FMPEP-d_2 in cortical regions is lower at baseline in chronic daily cannabis smokers (black bars, $n = 30$) than in control subjects (gray bars, $n = 28$). Values are estimated marginal means from the repeated measures analysis variance that controls for BMI. Values are adjusted to an average BMI of 24.8 kg m^{-2}. Error bars are s.e.m. Abbreviations: ACC, anterior cingulate cortex; AMY, amygdala; CAU, caudate nucleus; CER, cerebellum; HIPP, hippocampus; INS, insula; MIDBR, midbrain; OCC, occipital cortex; PAR, parietal cortex; PCC, posterior cingulate cortex; PFC, prefrontal cortex; PHIPP, parahippocampal gyrus; PUT, putamen; TEMP, lateral temporal cortex; THA, thalamus; VST, ventral striatum; WM, white matter; $*P<0.05$; $**P<0.005$, two-tailed t-test.

mapping analysis of voxel-wise V_T values, showing significantly lower cortical V_T in cannabis smokers (Figure 3). Among the cannabis smokers, years of cannabis smoking correlated negatively with V_T in cortical regions: subjects who had smoked cannabis for longer had smaller V_T than subjects who had smoked cannabis for a shorter time (Supplementary Figure 1). This correlation was not confounded by effects of age on V_T, because V_T did not correlate with age in control subjects. Current daily amounts of cannabis smoking or age of first cannabis smoking did not correlate with V_T.

In addition, V_T was not significantly correlated with withdrawal or craving measured on admission (for example, in the anterior cingulate cortex: withdrawal, $\rho = 0.06$, $P = 0.762$; craving, $\rho = -0.07$, $P = 0.773$) or as the mean value of these two scales for entire residential stay of $\sim$4 weeks (for example, in the anterior cingulate cortex: withdrawal, $\rho = -0.33$, $P = 0.077$; craving, $\rho = -0.05$, $P = 0.805$). In addition, the change of V_T during the residential stay was not significantly correlated with the change in withdrawal (for example, in the anterior cingulate cortex: $\rho = 0.04$, $P = 0.904$) or craving (for example, in the anterior cingulate cortex: $\rho = 0.23$, $P = 0.431$).

Ethnicity may affect V_T of [^{18}F]FMPEP-d_2, as three control subjects of Indian descent had unusually low V_T values, whereas no difference was found between African-American and Caucasian subjects of European descent (Supplementary Figure 2). There were no cannabis smokers of Indian descent. These three control subjects were not excluded from the analysis, but if they were excluded, the group $\times$ region interaction would become stronger ($F = 9.4$, $P = 0.0000002$).

BMI had a main effect on V_T in all regions ($F = 8.1$, $P = 0.006$); in both groups, higher BMI was associated with lower V_T. BMI did not correlate with the plasma free fraction of [^{18}F]FMPEP-d_2 in either group.

Only free (non-protein bound) radioligand in arterial plasma is able to enter the brain. However, smaller V_T in cannabis smokers than in control subjects was not caused by smaller fraction of free radioligand in arterial plasma, as this fraction was similar between groups (Table 1).

Tobacco smoking was more prevalent among cannabis smokers than among control subjects. However, a repeated measures analysis of variance did not show a main effect of tobacco smoking ($F < 0.01$, $P = 0.998$) or a tobacco smoking $\times$ region interaction ($F = 0.48$, $P = 0.735$) on V_T among cannabis smokers. In addition, when smoking was taken as a covariate in the overall model, the group $\times$ region interaction remained

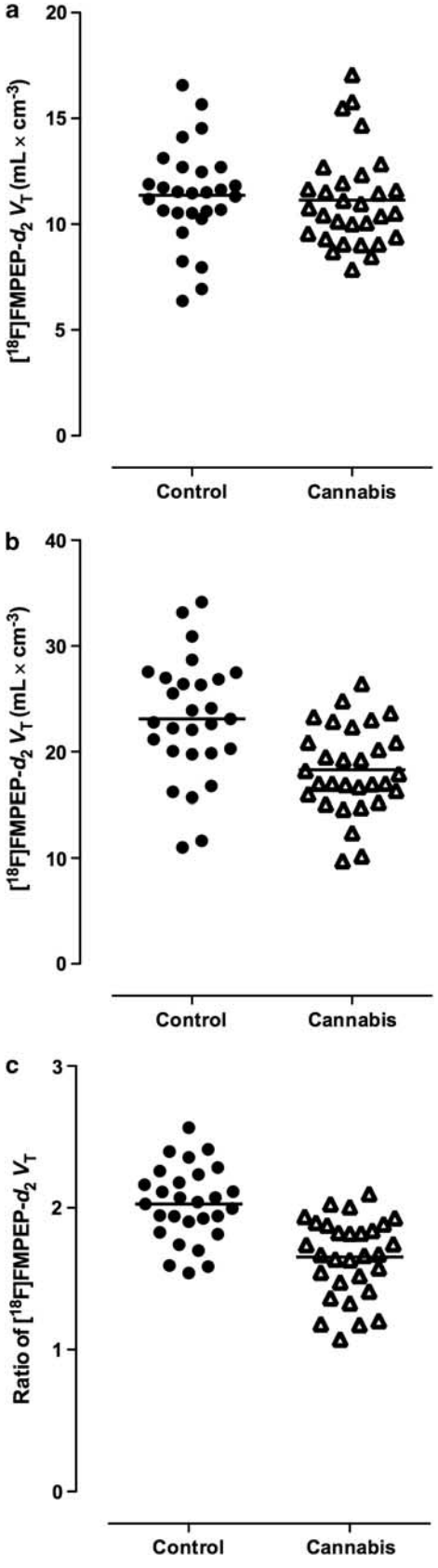

Figure 2 BMI-adjusted V_T of [^{18}F]FMPEP-d_2 at baseline was unchanged in cerebellum (−2%, $P = 0.703$, two-tailed *t*-test; **a**), but decreased in anterior cingulate cortex (−21%, $P = 0.0005$, two-tailed *t*-test; **b**) in chronic daily cannabis smokers ($n = 30$) compared with control subjects ($n = 28$). Ratio of V_T in anterior cingulate cortex to that in cerebellum decreased variance in both groups and showed a more significant reduction in cannabis smokers (−20%, $P = 0.0000001$, two-tailed *t*-test; **c**).

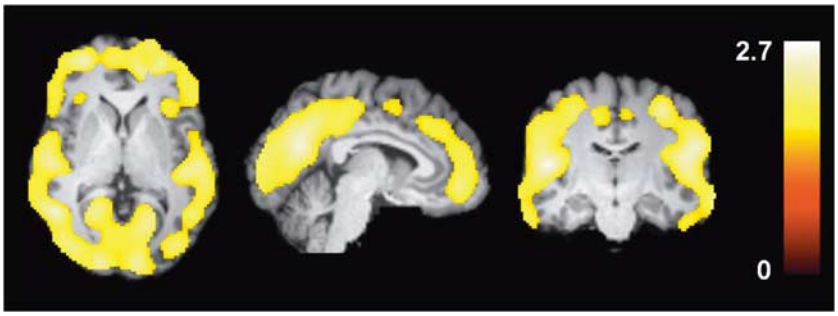

Figure 3 Statistical parametric mapping (SPM) analysis shows lower V_T in chronic daily cannabis smokers ($n = 30$) than in control subjects ($n = 28$) at baseline as a large single cluster that includes only cortical regions. This cluster comprised 67 513 voxels, had a maximum t-value of 2.8 at [−34, −78, 16] and had a cluster-level corrected P-value of 0.043. Color bar represents t-value in each voxel within the significant cluster.

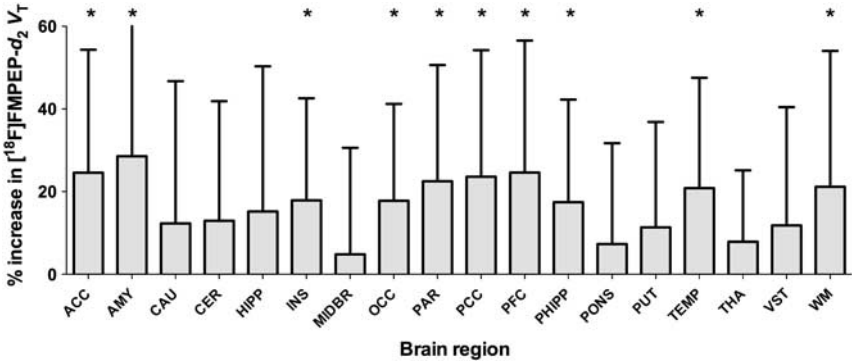

Figure 4 V_T of [^{18}F]FMPEP-d_2 increased after abstinence in the 14 cannabis smokers who completed two PET scans before and after abstinence. Significant increases were mostly seen in regions with reduced V_T at baseline. Error bars are s.d. of percent change. Abbreviations of brain regions are the same as those in Figure 1. *$P < 0.05$, two-tailed paired samples t-test.

significant ($F = 3.2$, $P = 0.017$). Similarly, when the analysis was covaried for alcohol use, the group-region interaction remained significant ($F = 6.6$, $P = 0.00009$). Thus, neither tobacco nor alcohol use were likely to significantly confound the main finding.

As V_T in cerebellum was similar between groups, we used cerebellum as a reference region to estimate binding in other regions of the brain. Although the majority of uptake in cerebellum represented specific binding, normalizing uptake in cortical regions to that in cerebellum for each subject was expected to reduce variability and to increase statistical power. For example, as BMI correlated with V_T in all regions, such normalization would cancel the effects of BMI. We found that uptake normalized to cerebellum was ~20% lower in cannabis smokers than in control subjects in cortical regions, but not in caudate nucleus, midbrain, thalamus or pons (group × region interaction: $F = 8.0$, $P = 0.0000002$).

To determine whether decreased V_T in cannabis smokers is reversible, we repeated PET measurements in 14 cannabis smokers after 26 ± 5 days of monitored abstinence (range 13–32 days). After monitored abstinence, V_T increased specifically in those brain regions that had shown decreased V_T at baseline (repetition × region interaction: $F = 3.33$, $P = 0.037$; Figure 4) except for hippocampus. At baseline, V_T was not different between subjects who had two PET scans ($N = 14$) and those who had one ($N = 16$; main effect of completer status: $F = 0.16$, $P = 0.691$; completer status × region interaction: $F = 0.43$, $P = 0.770$), which argues against selection bias. Change in V_T did not correlate with any of the clinical variables, such as current amount or years of cannabis smoking, or with the interval between the two PET scans. Fraction of free radioligand in arterial plasma did not change after abstinence ($P = 0.971$).

Discussion

We found that V_T of [^{18}F]FMPEP-d_2 at baseline was ~20% lower in chronic, daily cannabis smokers than in control subjects in cortical, but not in subcortical brain regions. V_T was also negatively correlated with years of cannabis smoking: subjects who had smoked cannabis longer had lower V_T than subjects who had smoked for a shorter time. Finally, V_T increased in cannabis smokers after ~4 weeks of abstinence. We interpret these findings as reflecting changes in CB_1 receptor density. Our findings are unaffected by group differences in peripheral distribution and metabolism

of the radioligand, because the outcome measure V_T corrects for these effects. These findings confirm that chronic cannabis exposure reversibly downregulates CB_1 receptors in humans, similar to its effects in rodents. Region-specific decrease in CB_1 receptor binding may be part of neuroadaptations promoting development of cannabis dependence in chronic daily smokers.

Decreased V_T in cannabis smokers likely reflects a change in CB_1 receptors (or affinity) rather than a confounding effect of an endogenous (for example, anandamide) or exogenous (for example, Δ^9-THC) agonist. Daily cannabis smokers have prolonged clearance of cannabinoids from the body,[31–33] and hence these subjects may have had cannabinoids present also in the brain during the first PET scan. Some PET radioligands are sensitive to changes in concentration of endogenous neurotransmitters, such that increased neurotransmitter concentration causes reduced binding of the radioligand.[34] Whether Δ^9-THC or other cannabinoid agonists compete with [^{18}F]FMPEP-d_2 in binding to CB_1 receptors in human brain remains to be established, although such occupancy would not be expected to be restricted to cortical brain regions. In rat brain, binding of [^{11}C]MePPEP, a close analog to [^{18}F]FMPEP-d_2, was not displaced by high doses of cannabinoid agonists,[35] consistent with large receptor reserve. Nevertheless, CB_1 receptor occupancy by Δ^9-THC or its metabolites remains a potential confound. Another potential confound is a change in G-protein coupling of the CB_1 receptor (desensitization), which has been documented in rodent studies. The radioligand [^{18}F]FMPEP-d_2 is an inverse agonist and presumably labels receptors in both high- and low-affinity states, as binding of another structurally related inverse agonist, rimonabant, is not affected by guanyl nucleotide modulation in rat brain tissue *in vitro*.[36] Therefore, [^{18}F]FMPEP-d_2 is unlikely to detect CB_1 receptor desensitization that occurs after chronic agonist exposure in rodents.[15]

Although our results suggest changes in CB_1 receptor density, our PET measurement cannot distinguish a change in receptor density and affinity. However, rodent studies suggest a change in receptor density rather than affinity. Our findings of reversible and regionally selective downregulation of CB_1 receptors parallel those from a large number of rodents studies.[15] These studies demonstrated substantial downregulation of CB_1 receptors because of loss of CB_1 receptor protein after chronic agonist exposure.[15,20] One post-mortem study in humans also reported CB_1 receptor downregulation in cannabis smokers that was greatest in hippocampus and least in globus pallidus and substantia nigra.[37] The regional selectivity of downregulation is strikingly similar between rodents and humans in the current study: no significant downregulation was found in the basal ganglia and the midbrain in humans. Furthermore, we did not find downregulation in cerebellum, which is consistent with rodent studies showing less downregulation in this area compared with hippocampus.[16,21] The reasons for regional selectivity are unclear, but rodent studies suggest regional differences in distribution of markers of CB_1 receptor signaling, such as G-protein subunits, subtypes of adenylyl cyclase, receptor kinases, arrestins and other proteins associated with receptor signaling.[15,20,38]

Could CB_1 receptor downregulation be a mechanism for tolerance to cannabis in humans? That is, could regional differences in receptor downregulation predict differential development of tolerance toward various effects of cannabis? Interestingly, chronic daily cannabis smokers are tolerant to most, but not all, effects of cannabis. For example, tolerance develops for memory impairment,[4,5] but not for feeling of high[4,5] or motor impairment,[5] induced by both intravenous and smoked Δ^9-THC. Feelings of high and motor impairment might be driven by CB_1 receptors in the basal ganglia, midbrain and cerebellum—regions that did not show receptor downregulation in the current study. Another interesting finding is that hippocampus did not show reversal of downregulation after abstinence: prolonged downregulation might contribute to long-term cognitive impairment in chronic daily cannabis smokers noted by some studies.[39] This hypothesis, however, is simplistic because CB_1 receptors are widespread in the human brain and complex psychological effects of cannabis are probably not limited to receptors in one particular brain region. In addition, CB_1 receptor desensitization, which we cannot measure, may still occur in these regions despite unchanged receptor density. Finally, we did not objectively measure tolerance to cannabis. We only measured withdrawal and craving, neither of which correlated with CB_1 receptor binding. Future studies should establish the relationship between tolerance to cannabis and regional CB_1 receptor downregulation.

We found decreased CB_1 receptor binding in subjects who had smoked large amounts of cannabis daily for years. Even in these heavy smokers, binding returned to normal levels in most regions after $\sim$4 weeks of abstinence. We are unsure whether moderate and occasional cannabis smoking would produce similar downregulation. Considering that occasional cannabis smokers do not develop tolerance to the extent that chronic daily smokers do,[40] downregulation of CB_1 receptors may be smaller in occasional smokers, assuming that receptor downregulation contributes to tolerance. Nevertheless, future studies should specifically address that question by measuring CB_1 receptor binding in occasional cannabis smokers.

Tobacco smoking was more prevalent among cannabis smokers than among control subjects. However, tobacco smoking is unlikely to explain the region-specific and reversible decrease in CB_1 receptor binding in cannabis smokers, because tobacco smoking had no effects on V_T in cannabis smokers or on the group $\times$ region interaction in the overall model. In addition, V_T increased after $\sim$4 weeks of abstinence

from cannabis, even though subjects continued smoking tobacco during that time. Nevertheless, future studies should examine the effects of tobacco smoking on CB_1 receptor binding in control subjects who do not smoke cannabis.

BMI correlated negatively with [^{18}F]FMPEP-d_2 V_T in both groups, such that people with higher BMI tended to have lower V_T than people with lower BMI. Although the cause of this association is unclear, we doubt that it represents an association with CB_1 receptor density. Brain CB_1 receptors are implicated in feeding behaviors.[41] CB_1 receptor stimulation in mouse brain can both promote and inhibit feeding behavior, depending on the dose of the agonist and the brain region involved.[42] However, we found similar correlations in all brain regions, not restricted to those implicated in feeding behavior. Therefore, we suspect that this correlation is driven by some peripheral confound—potentially the fraction of free radioligand in plasma. For [^{18}F]FMPEP-d_2, this fraction is very small ($<1\%$) and imprecisely measured.[22] Although we found no correlation between BMI and the measured plasma free fraction, a small but significant correlation cannot be excluded. The primary finding of decreased V_T in cannabis smokers was region specific and therefore not likely confounded by the plasma free fraction that would affect V_T globally. Of note, the outcome measure V_T corrects for peripheral metabolism and disposition of the radioligand, which makes these factors unlikely confounders. The important implication of this finding is that BMI should be taken as a covariate in [^{18}F]FMPEP-d_2 studies, whatever the underlying mechanism.

In conclusion, we report for the first time decreased CB_1 receptor binding in cortical, but not in subcortical, brain regions in chronic daily cannabis smokers, and that this downregulation is reversible after $\sim$4 weeks of abstinence. Downregulation of CB_1 receptors may underlie tolerance to effects of cannabis, and may provide insight into the role of CB_1 receptors in diseases that are associated with chronic cannabis smoking, such as psychosis and depression. Future studies should determine whether occasional cannabis smokers or females show similar downregulation, and examine the time course of reversal of downregulation in greater detail.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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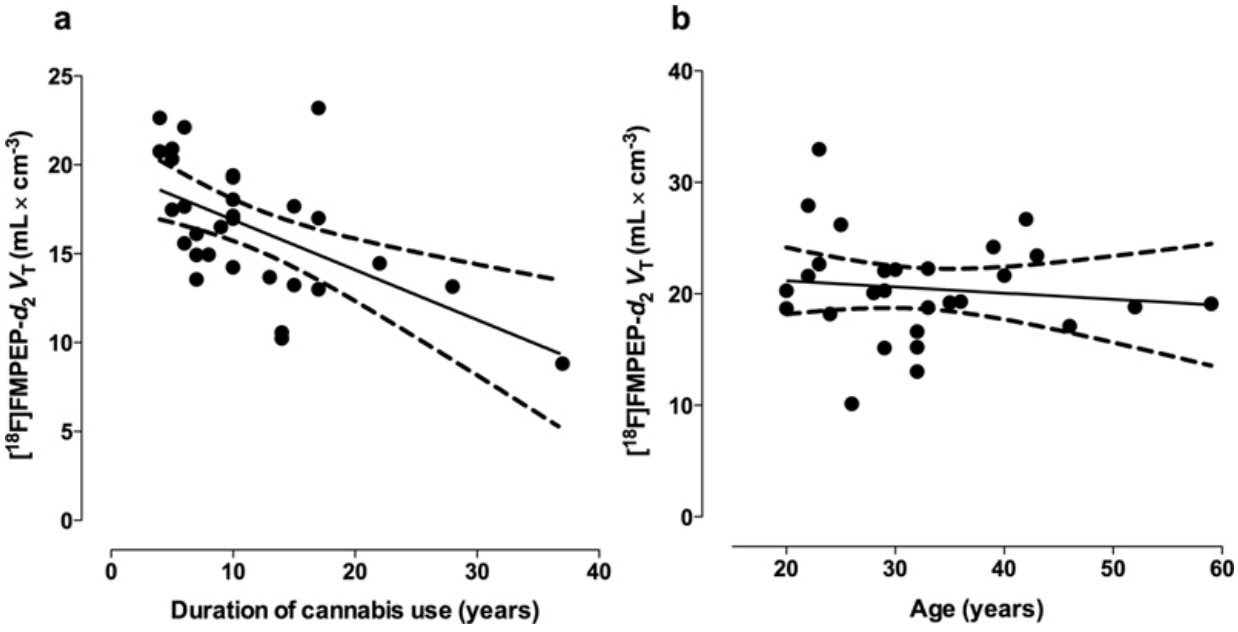
References

1 Degenhardt L, Chiu WT, Sampson N, Kessler RC, Anthony JC, Angermeyer M *et al.* Toward a global view of alcohol, tobacco, cannabis, and cocaine use: findings from the WHO World Mental Health Surveys. *PLoS Med* 2008; **5**: e141.
2 Hall W, Degenhardt L. Adverse health effects of non-medical cannabis use. *Lancet* 2009; **374**: 1383–1391.
3 Jones RT, Benowitz NL, Herning RI. Clinical relevance of cannabis tolerance and dependence. *J Clin Pharmacol* 1981; **21**: 143S–152S.
4 D'Souza DC, Ranganathan M, Braley G, Gueorguieva R, Zimolo Z, Cooper T *et al.* Blunted psychotomimetic and amnestic effects of Δ-9-tetrahydrocannabinol in frequent users of cannabis. *Neuropsychopharmacology* 2008; **33**: 2505–2516.
5 Ramaekers JG, Kauert G, Theunissen EL, Toennes SW, Moeller MR. Neurocognitive performance during acute THC intoxication in heavy and occasional cannabis users. *J Psychopharmacol* 2009; **23**: 266–277.
6 Haney M, Ward AS, Comer SD, Foltin RW, Fischman MW. Abstinence symptoms following smoked marijuana in humans. *Psychopharmacology (Berl)* 1999; **141**: 395–404.
7 Cooper ZD, Haney M. Actions of delta-9-tetrahydrocannabinol in cannabis: relation to use, abuse, dependence. *Int Rev Psychiatry* 2009; **21**: 104–112.
8 Huestis MA, Gorelick DA, Heishman SJ, Preston KL, Nelson RA, Moolchan ET *et al.* Blockade of effects of smoked marijuana by the CB1-selective cannabinoid receptor antagonist SR141716. *Arch Gen Psychiatry* 2001; **58**: 322–328.
9 Huestis MA, Boyd SJ, Heishman SJ, Preston KL, Bonnet D, Le Fur G *et al.* Single and multiple doses of rimonabant antagonize acute effects of smoked cannabis in male cannabis users. *Psychopharmacology (Berl)* 2007; **194**: 505–515.
10 Herkenham M, Lynn AB, Little MD, Johnson MR, Melvin LS, de Costa BR *et al.* Cannabinoid receptor localization in brain. *Proc Natl Acad Sci USA* 1990; **87**: 1932–1936.
11 Glass M, Dragunow M, Faull RL. Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. *Neuroscience* 1997; **77**: 299–318.
12 Howlett AC, Barth F, Bonner TI, Cabral G, Casellas P, Devane WA *et al.* International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacol Rev* 2002; **54**: 161–202.
13 Wilson RI, Nicoll RA. Endocannabinoid signaling in the brain. *Science* 2002; **296**: 678–682.
14 Gonzalez S, Cebeira M, Fernandez-Ruiz J. Cannabinoid tolerance and dependence: a review of studies in laboratory animals. *Pharmacol Biochem Behav* 2005; **81**: 300–318.
15 Sim-Selley LJ. Regulation of cannabinoid CB1 receptors in the central nervous system by chronic cannabinoids. *Crit Rev Neurobiol* 2003; **15**: 91–119.
16 Breivogel CS, Childers SR, Deadwyler SA, Hampson RE, Vogt LJ, Sim-Selley LJ. Chronic delta9-tetrahydrocannabinol treatment produces a time-dependent loss of cannabinoid receptors and cannabinoid receptor-activated G proteins in rat brain. *J Neurochem* 1999; **73**: 2447–2459.

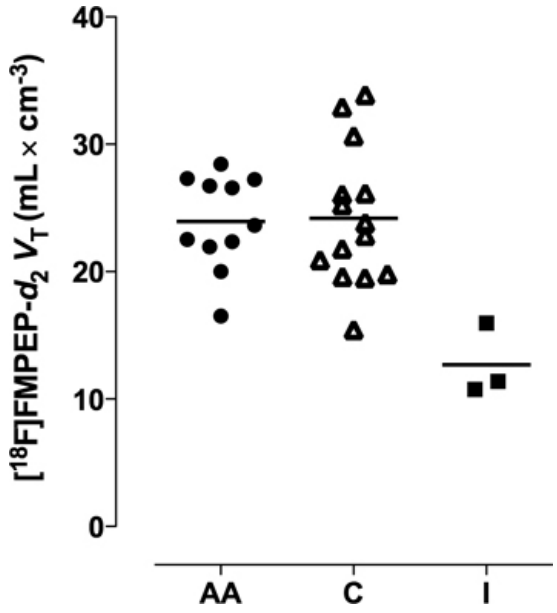
17 Oviedo A, Glowa J, Herkenham M. Chronic cannabinoid administration alters cannabinoid receptor binding in rat brain: a quantitative autoradiographic study. *Brain Res* 1993; **616**: 293–302.
18 Sim-Selley LJ, Martin BR. Effect of chronic administration of R-(+)-[2,3-Dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo[1,2,3-de]-1,4-benzoxazinyl]-(1-naphthalenyl)methanone mesylate (WIN55,212-2) or delta 9-tetrahydrocannabinol on cannabinoid receptor adaptation in mice. *J Pharmacol Exp Ther* 2002; **303**: 36–44.
19 Breivogel C, Scates SM, Beletskaya IO, Lowery OB, Aceto MD, Martin BR. The effects of Δ9-tetrahydrocannabinol physical dependence on brain cannabinoid receptors. *Eur J Pharmacol* 2003; **459**: 139–150.
20 Sim-Selley LJ, Schechter NS, Rorrer WK, Dalton GD, Hernandez J, Martin BR *et al.* Prolonged recovery rate of CB1 receptor adaptation after cessation of long-term cannabinoid administration. *Mol Pharmacol* 2006; **70**: 986–996.
21 McKinney DL, Cassidy MP, Collier LM, Martin BR, Wiley JL, Selley DE *et al.* Dose-related differences in the regional pattern of cannabinoid receptor adaptation and in vivo tolerance development to 9-tetrahydrocannabinol. *J Pharmacol Exp Ther* 2008; **324**: 664–673.
22 Terry GE, Hirvonen J, Liow JS, Zoghbi SS, Gladding R, Tauscher JT *et al.* Imaging and quantitation of cannabinoid CB1 receptors in human and monkey brains using (18)F-labeled inverse agonist radioligands. *J Nucl Med* 2010; **51**: 112–120.
23 Donohue SR, Krushinski JH, Pike VW, Chernet E, Phebus L, Chesterfield AK *et al.* Synthesis, ex vivo evaluation, and radiolabeling of potent 1,5-diphenylpyrrolidin-2-one cannabinoid subtype-1 receptor ligands as candidates for in vivo imaging. *J Med Chem* 2008; **51**: 5833–5842.
24 Heishman SJ, Singleton EG, Liguori A. Marijuana craving questionnaire: development and initial validation of a self-report instrument. *Addiction* 2001; **96**: 1023–1034.
25 Zoghbi SS, Shetty HU, Ichise M, Fujita M, Imaizumi M, Liow JS *et al.* PET imaging of the dopamine transporter with 18F-FECNT: a polar radiometabolite confounds brain radioligand measurements. *J Nucl Med* 2006; **47**: 520–527.
26 Gandelman MS, Baldwin RM, Zoghbi SS, Zea-Ponce Y, Innis RB. Evaluation of ultrafiltration for the free-fraction determination of single photon emission computed tomography (SPECT) radiotracers: beta-CIT, IBF, and iomazenil. *J Pharm Sci* 1994; **83**: 1014–1019.
27 Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N *et al.* Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage* 2002; **15**: 273–289.
28 Burger C, Mikolajczyk K, Grodzki M, Rudnicki P, Szabatin M, Buck A. Java tools for quantitative post-processing of brain PET data. *J Nucl Med* 1998; **39**: 277–278.
29 Friston KJ, Holmes AP, Worsley KJ, Poline JP, Frith C, Frackowiak RSJ. Statistical parametric maps in functional imaging: a general linear approach. *Hum Brain Mapp* 1995; **2**: 189–210.
30 Innis RB, Cunningham VJ, Delforge J, Fujita M, Gjedde A, Gunn RN *et al.* Consensus nomenclature for in vivo imaging of reversibly binding radioligands. *J Cereb Blood Flow Metab* 2007; **27**: 1533–1539.
31 Karschner EL, Schwilke EW, Lowe RH, Darwin WD, Herning RI, Cadet JL *et al.* Implications of plasma Delta9-tetrahydrocannabinol, 11-hydroxy-THC, and 11-nor-9-carboxy-THC concentrations in chronic cannabis smokers. *J Anal Toxicol* 2009; **33**: 469–477.
32 Karschner EL, Schwilke EW, Lowe RH, Darwin WD, Pope HG, Herning R *et al.* Do Delta9-tetrahydrocannabinol concentrations indicate recent use in chronic cannabis users? *Addiction* 2009; **104**: 2041–2048.
33 Lowe RH, Abraham TT, Darwin WD, Herning R, Cadet JL, Huestis MA. Extended urinary Delta9-tetrahydrocannabinol excretion in chronic cannabis users precludes use as a biomarker of new drug exposure. *Drug Alcohol Depend* 2009; **105**: 24–32.
34 Innis RB, Malison RT, al-Tikriti M, Hoffer PB, Sybirska EH, Seibyl JP *et al.* Amphetamine-stimulated dopamine release competes in vivo for [123I]IBZM binding to the D2 receptor in nonhuman primates. *Synapse* 1992; **10**: 177–184.
35 Terry G, Liow J, Chernet E, Zoghbi S, Phebus L, Felder C *et al.* Positron emission tomography imaging using an inverse agonist radioligand to assess cannabinoid CB1 receptors in rodents. *Neuroimage* 2008; **41**: 690–698.
36 Rinaldi-Carmona M, Pialot F, Congy C, Redon E, Barth F, Bachy A *et al.* Characterization and distribution of binding sites for [3H]-SR 141716A, a selective brain (CB1) cannabinoid receptor antagonist, in rodent brain. *Life Sci* 1996; **58**: 1239–1247.
37 Villares J. Chronic use of marijuana decreases cannabinoid receptor binding and mRNA expression in the human brain. *Neuroscience* 2007; **145**: 323–334.
38 Martin BR, Sim-Selley LJ, Selley DE. Signaling pathways involved in the development of cannabinoid tolerance. *Trends Pharmacol Sci* 2004; **25**: 325–330.
39 D'Souza DC. Cannabinoids and psychosis. *Int Rev Neurobiol* 2007; **78**: 289–326.
40 Cooper ZD, Haney M. Cannabis reinforcement and dependence: role of the cannabinoid CB1 receptor. *Addict Biol* 2008; **13**: 188–195.
41 Cota D. CB1 receptors: emerging evidence for central and peripheral mechanisms that regulate energy balance, metabolism, and cardiovascular health. *Diabetes Metab Res Rev* 2007; **23**: 507–517.
42 Bellocchio L, Lafenêtre P, Cannich A, Cota D, Puente N, Grandes P *et al.* Bimodal control of stimulated food intake by the endocannabinoid system. *Nat Neurosci* 2010; **13**: 281–283.

Supplementary Information accompanies the paper on the Molecular Psychiatry website (http://www.nature.com/mp)

Supplementary Figure 1. BMI-adjusted V_T of [^{18}F]FMPEP-d_2 at baseline in cannabis smokers (n=30) correlated negatively with years of cannabis smoking in prefrontal cortex (ρ=-0.55, p=0.002) (A). This correlation could be confounded by age. No correlation, however, was seen between BMI-adjusted V_T of [^{18}F]FMPEP-d_2 and age in control subjects (n=28, R=-0.10, p=0.615) (B). Prefrontal cortex is representative of all neocortical regions. Curved dashed lines are bounds for the 95% confident interval for a linear regression fit for visualization purposes.



Supplementary Figure 2. Control subjects of Indian descent had lower BMI-adjusted V_T of [^{18}F]FMPEP-d_2 in prefrontal cortex than other control subjects. Prefrontal cortex is representative of all brain regions. AA, African-American; C, Caucasian of European descent; I, Indian.



Supplementary Methods

Positron emission tomography

PET images were spatially normalized to a ligand-specific template using Statistical Parametric Mapping package (SPM5)[1] running on Matlab (version 7.2.0.232, Mathworks, Natick, MA) by estimating parameters from time-averaged PET images. The ligand-specific template was created in-house using PET images from twelve healthy subjects. Regional radioactivity concentration was measured from the spatially normalized dynamic PET images and the Automated Anatomical Labeling template of volumes of interest[2] as implemented in PMOD, version 3.0 (PMOD Technologies Ltd)[3]. Data from the Automated Anatomical Labeling template were consolidated by volume-weighted averaging into the following volumes of interest: anterior cingulate cortex, amygdala, caudate, cerebellum, hippocampus, insula, occipital cortex, parietal cortex, posterior cingulate cortex, parahippocampal gyrus, putamen, lateral temporal cortex, and thalamus. The following volumes of interest were manually added to the template: midbrain, pons, ventral striatum, and white matter. Ventral striatum was delineated onto coronal sections according to a published guideline[4]. Distribution volume (V_T) was estimated according to the 2-tissue compartmental model[5] with concentration of parent radioligand in plasma as input function, using PMOD, as previously described[6].

Voxel-wise analysis of V_T

To confirm results from volume of interest analysis and to explore regional specificity of findings, we compared baseline parametric V_T maps between groups at voxel-level using SPM5. Parametric V_T images were calculated using the Logan plot with arterial input function as implemented in PMOD. V_T images were then smoothed

with a 12-mm Gaussian kernel (full width at half maximum), and tested between groups at baseline using a two-sample t-test with BMI as covariate in SPM5. No global normalization was applied. Cluster-level corrected p-values smaller than 0.05 were considered statistically significant.

References

1. Friston, K.J., Holmes, A.P., Worsley, K.J., Poline, J.P., Frith, C. & Frackowiak, R.S.J. Statistical parametric maps in functional imaging: a general linear approach. *Human Brain Mapping* 1995; **2**, 189-210.
2. Tzourio-Mazoyer, N., Landeau, B., Papathanassiou, D., Crivello, F., Etard, O., Delcroix, N. *et al.* Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage* 2002; **15**, 273-289.
3. Burger, C., Mikolajczyk, K., Grodzki, M., Rudnicki, P., Szabatin, M. & Buck, A. Java tools for quantitative post-processing of brain PET data. *Journal of Nuclear Medicine* 1998; **39**, 277p-278p.
4. Mawlawi, O., Martinez, D., Slifstein, M., Broft, A., Chatterjee, R., Hwang, D.R. *et al.* Imaging human mesolimbic dopamine transmission with positron emission tomography: I. Accuracy and precision of D(2) receptor parameter measurements in ventral striatum. *J Cereb Blood Flow Metab* 2001; **21**, 1034-1057.
5. Innis, R.B., Cunningham, V.J., Delforge, J., Fujita, M., Gjedde, A., Gunn, R.N. *et al.* Consensus nomenclature for in vivo imaging of reversibly binding radioligands. *J Cereb Blood Flow Metab* 2007; **27**, 1533-1539.

6. Terry, G.E., Hirvonen, J., Liow, J.S., Zoghbi, S.S., Gladding, R., Tauscher, J.T. *et al.* Imaging and quantitation of cannabinoid CB1 receptors in human and monkey brains using (18)F-labeled inverse agonist radioligands. *J Nucl Med* 2010; **51**, 112-120.